AD________________

Award Number: DAMD17-99-1-9136

TITLE: Adhesion-Dependent Regulation of Cell Growth and Apoptosis in Human Breast Cancer

PRINCIPAL INVESTIGATOR: David M. Helfman, Ph.D.

CONTRACTING ORGANIZATION: Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

REPORT DATE: August 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012



20030130 198

# REPORT DOCUMENTATION PAGE

*Form Approved OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | August 2002 | Final (1 Jul 99 - 1 Jul 02) |

**4. TITLE AND SUBTITLE**
Adhesion-Dependent Regulation of Cell Growth and Apoptosis in Human Breast Cancer

**5. FUNDING NUMBERS**
DAMD17-99-1-9136

**6. AUTHOR(S)**
David M. Helfman, Ph.D.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

**E-Mail:** helfman@cshl.org

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200 Words)*

We have studied the role of the actin cytoskeleton and myosin II in adhesion-dependent signaling and regulation of apoptosis in normal and transformed epithelial cells. Normal epithelial cells require attachment to the extracellular matrix (ECM) for survival, and disruption of cell-ECM interactions results in induction of apoptosis, a phenomenon termed "anoikis". By contrast, transformed cells do not require interaction with the ECM and do not undergo apoptosis when grown in suspension. This property plays a critical role in the ability of cancerous cells to metastasize. We have found that activation of myosin II is a critical step in the generation of signals that prevent programmed cell death. In addition, these studies show that transformed epithelial "escape" apoptosis due to constitutive activation of signaling pathways dependent on myosin II. We have identified key-signaling pathways that are altered in transformed cells and thereby contribute to the ability of tumor cells to escape anoikis. Collectively these studies provide important new information regarding specific pathways that regulate myosin II and identify potential therapeutic targets for the treatment of breast cancer, as well as other cancers.

**14. SUBJECT TERMS**
breast cancer, epithelial cells, extracellular matrix (ECM), apoptosis, anoikis, myosin, cytoskeleton, actin

**15. NUMBER OF PAGES**
16

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

## Table of Contents

## INTRODUCTION:

The goal of this research program was to better understand the molecular basis of metastasis. The ability of a tumor cell to grow outside its local environment (metastasize) is a major problem in the development and therapeutic treatment of cancer. Adhesion of cells to the extracellular matrix (ECM) and neighboring cells plays a critical role in various cellular processes linked to transformation including differentiation, growth, motility and programmed cell death (apoptosis). Regulation of cell death is an essential component in the body's defense against the emergence of cancer. Attachment of cells to the ECM is important for the generation of signals that regulate normal cell proliferation and apoptosis. The loss of the requirement for cell-matrix interactions plays a critical role in the development of cancer. Recent experiments by us and other laboratories have shown that activation of myosin II is an essential step during adhesion-mediated signal transduction. In addition, this suggests a mechanism by which changes in cellular proteins involved in the regulation of myosin II function will lead to aberrant growth control by constitutively activating downstream signaling pathways. The experiments outlined in this grant tested the hypothesis that constitutive activation of myosin II contributes, in part, to the transformed phenotype and to the inability of transformed cells to undergo apoptosis. Specifically, we asked if inhibition of myosin results in activation of the apoptotic pathway in normal and transformed cells. Our studies have provide important new information on the role of the actin cytoskeleton and myosin II in adhesion-dependent cell signaling and the potential of inhibiting signal transduction pathways dependent on myosin II as a therapeutic target and adjuvant for the treatment of breast cancer, as well as other cancers.

## BODY:

The observation that activation of myosin II is an essential step during adhesion-mediated signal transduction suggests a mechanism by which changes in cellular proteins involved in the regulation of myosin function in transformed cells will contribute to aberrant growth control. The experiments outlined for this grant in the original "**Statement of Work"** included experiments to analyze the effects of treating cells with various agents to determine if inhibition of myosin II or disruption of the actin cytoskeleton induces apoptosis. These experiments were designed to test the hypothesis that constitutive activation of myosin II contributes, in part, to the transformed phenotype and to the insensitivity of transformed cells to undergo apoptosis. Accordingly, during this funding period we determined if inhibition of myosin, or disruption of the actin cytoskeleton results in activation of the apoptotic pathway in adhesion-independent cells. If activation of myosin II is a critical step in the pathway of adhesion-dependent suppression of apoptosis, then inhibiting myosin function will promote apoptosis. These studies provided important new information concerning the role of actin filaments and myosin in adhesion-dependent cell signaling and the potential of inhibiting signal transduction pathways dependent on actomyosin contractility as a therapeutic target and adjuvant for the treatment of breast cancer, as well as other cancers.

## KEY RESEARCH ACCOMPLISHMENTS:

The experiments conducted during the funding period were designed to determine if activation of myosin II (actomyosin contractility) plays a role in intracellular signaling following cell-ECM interactions and thereby prevents apoptosis. Accordingly, if loss of cell-ECM interactions results in a decrease in actomyosin contractility and thereby activates the apoptotic pathway in normal cells, then inhibition of myosin itself should lead to apoptosis. In addition, if transformed cells are able to exhibit adhesion-independent cell growth because of constitutive activation of myosin II, then inhibiting myosin II function should effect cell growth and cell survival. Accordingly, we studied the effects of altering myosin II function in normal MDCK cells and MDCK cells transformed with the ras oncogene, and a number of other transformed human epithelial cells.

We used three different compounds that inhibit myosin II, namely Butanedione monoxime (BDM ), ML-7, and Y27632. BDM inhibits the ATPase activity of myosin, ML-7 blocks myosin light chain kinase and thereby inhibits myosin II contractility, and Y27632, which inhibits Rho-kinase 9ROCK), which is a regulator of myosin II. Apoptosis was be assayed by staining cells with DAPI and viewing the nuclei or by analysis of DNA degradation. We also assayed for other known markers of apoptosis including staining cells for annexin V, and processing of caspases. For the studies we continually obtained the advice of Drs. Yuri Lazebnick, and Scott Lowe, at Cold Spring Harbor Laboratory, who are experts in apoptosis. We treated cells with 2,3-butanemonoxime (BDM) and ML-7 (Zhong et al., 1997), both of which have been shown to inhibit the function of myosin II contractility. BDM inhibits the release of ATP from the myosin head, and therefore inhibits binding of myosin to actin, and ML-7 inhibits MLCK. Y27632 inhibits ROCK, which is a protein kinase that can phosphorylate the myosin light chain and also inhibit this myosin phosphatase, the net result is an increase in myosin II activity. Latrunculin A and cytochalasin D were also used to determine the role of actin inhibition on cell death. Latrunculin A binds to actin monomers and prevents polymerization of actin. Cytochalasin D binds to actin filaments preventing association or dissociation from that end (Brown and Spudich, 1981). We have also used the general caspase inhibitor zVAD-FMK to inhibit activation of caspases in treated cells. Cells treated with this compound should resemble untreated cells i.e, no caspase activation. Cells were treated for various time points and concentrations to determine desired amount of cell death.

### Treatment of normal and transformed cells with myosin inhibitors BDM and ML-7 but not Y27632 results in apoptosis and activation of caspase 8

MDCK, MDCK Ras, and MCF-7 cells were treated with BDM, ML-7 or Y27632 to determine the importance of myosin II in these cells. They were tested for the presence of an activated caspase. Activated caspases are cleaved and of smaller molecular weight than the inactive form. Activation of caspase-8 was examined because this is an initiator caspase. An overnight treatment of cells with 50mM BDM was harvested, a whole cell extract was prepared, and extracts were run on a 10% SDS gel. This was transferred to nitrocellulose, and blots were probed with a caspase-8 monoclonal antibody, which recognizes inactive caspase-8 (55kD) and active caspase–8 (45kD). The activation of caspase-8 with myosin inhibition in MDCK and MDCK Ras cells was detected. zVAD-FMK, a general caspase inhibitor, was used to test for inhibition of caspase-8 activation in treated cells. Treatment of cells with this caspase inhibitor led to the disappearance of the activated caspase-8. Similar results were observed following myosin inhibition with BDM in MDCK Ras cells. In addition, MDCK cells treated with another myosin inhibitor, ML7 also exhibited activation of caspase-8. Similar results were found with activation of caspase-8 in the human breast cancer cell line MCF7. By contrast, treatment of cells with the Rho-kinase inhibitor, Y27632 did not induce apoptosis in either normal or transformed epithelial cells. These results lead to two important conclusions: (1) that myosin II plays a critical role in the role in the generation of signals that prevent apoptosis in both normal and transformed cells, and 2) myosin

light chain kinase, and not Rho-kinase, plays a critical role in the regulation of myosin II-dependent in signals that prevent apoptosis.

**Disruption of the actin cytoskeleton in normal and transformed cells results in apoptosis and activation of caspase 8**

Cells were also treated with latrunculin A, a compound that binds to actin monomers preventing polymerization, and cytochalasin D, a compound that binds to the barbed ends of actin filaments, preventing further polymerization. After an overnight treatment of MDCK cells with either latrunculin or cytochalasin D, cells were extracted, run on a 10% gel and transferred to a nitrocellulose membrane. The blots were probed with anti caspase-8 antibody to determine induction of apoptosis. With both latrunculin A and cytochalasin D caspase-8 is activated, and activation is inhibited with zVAD-FMK. These results demonstrate the induction of apoptosis in cells where actomyosin contractility is inhibited. Apoptosis is characterized by many morphological changes, including chromatin condensation, cell shrinkage, and membrane blebbing (Thornberry and Lazebnik, 1998). To confirm apoptosis in cells treated with inhibitors of myosin or actin, MDCK cells were grown on coverslips, treated with varying concentrations of BDM after 24 or 48 hours, then fixed with 3% paraformaldehyde and permeablized with 0.1% Triton. The cells were then stained with DAPI, which binds to cell DNA. Examination of the coverslips on a fluorescent microscope revealed the presence of many condensed nuclei (data not shown). Similar results were found with treatment of MDCK cells with ML7, latrunculin A and cytochalasin D (data not shown).

Table I below summarizes the data from our analyses. As indicated in Table I, when normal MDCK cells were plated on poly-HEMA approximately 50% of the cells underwent programmed cells death, in agreement with previous studies showing these cells undego apoptosis when deprived of the substratum (Frisch et al., 1994). By contrast the human breast cancer cell line MCF7 was relatively resistant to loss of matrix interactions. However, treatment of normal or transformed cells with inhibitors of myosin or agents that disrupt the actin cytoskeleton resulted in induction of apoptosis. For example, treatment of the MCF7 cells with BDM or ML7 resulted in apoptosis in 60-70% of the cells. These results are in agreement with our hypothesis that activation of actomyosin contractility is a critical step in the generation of signals that prevent apoptosis. In addition, the same results were obtained using two different agents to inhibit myosin, namely BDM and ML7 that have different mechanisms of actin to inhibit myosin II activity. Butanedione monoxime (BDM) inhibits the ATPase activity of myosin, and ML7 blocks myosin light chain kinase and thereby inhibits myosin II contractility. In addition, disruption of the cytoskeleton by latrunculin A or cytochalasin D also resulted in a larger percentage of cells undergoing apoptosis compared to cells plated on polyHEMA. The induction of apoptosis by the various pharmacological agents was not simply due to disruption of adhesion of cells to the substratum, because identical results were obtained when cells were analyzed following fixation and staining for DAPI to score cells for chromosome condenstation .

**Table 1: % Living Cells After Overnight Treatment**

| | Normal Untreated | Polyhema | 40mM BDM | 30uM CD | 1.0 um Lat A | 40uM ML7 |
|---|---|---|---|---|---|---|
| MDCK | 85 | 52 | 45 | 52 | 43 | 36 |
| MDCK Ras | 95 | 94 | 58 | 75 | 50 | 58 |
| MCF7 | 92 | 71 | 62 | - | - | 54 |
| DU145 | 91 | 76 | 53 | - | - | 47 |

In agreement with our hypothesis, inhibition of actomyosin contractility causes apoptosis in both normal and transformed epithelial cells. Using inhibitors of myosin and actin function, we have demonstrated that normal and transformed epithelial cells will undergo apoptosis. This is demonstrated with the presence of activated caspases, is shown clearly by the presence of chromatin condensation, and by staining cells for apoptotic markers.

The results of our data indicate that agents that inhibit myosin II function lead to programed cell death in epithelial cells. Thus, actomyosin contractility plays a critical role in the generation of signals required for the prevention of apoptosis in epithelial cells (Pawlak and Helfman, 2001 [Appendix]).

**REPORTABLE OUTCOMES:**

This funding provides research support for a doctoral student and a postdoctoral fellow. We are currently in the process of preparing a manuscript of these studies, which we plan to submit by September 2002.

**Cytoskeletal changes in cell transformation and tumorigenesis.** Geraldine Pawlak and David M. Helfman. *Current Opinion in Genetics & Development* 2001, 11:41-47.

**Regulation of apoptosis by myosin II in normal and transformed cells**. Laureen E. Connell and David M. Helfman. Manuscript in Preparation.

**CONCLUSIONS:**

The results of our studies are in agreement of our hypothesis concerning the role myosin II function plays a critical role in adhesion-dependent cell growth and in the apoptotic response of cells. In this regard it is interesting to note that Rho plays a role in both transformation (Qiu et al., 1995) and metastasis (Yoshioka et al, 1998). Rho is a downstream effector of the ras pathway is known to stimulate the actomyosin system and this is thought to play a direct role in invasion of tumor cells (Yoshioka et al., 1998). While myosin II is believed to play a critical role in the motility of tumor cells, our studies demonstrate that activation of myosin II activates signal transduction pathways associated with adhesion-dependent cell growth, that antagonize the normal apoptotic pathway and lead to survival of tumor cells outside their normal environment. The studies obtained from the current grant have provided two important new insights into abnormal growth control; (1) that myosin II plays a critical role in the role in the generation of signals that prevent apoptosis in both normal and transformed cells, and 2) myosin light chain kinase, and not Rho-kinase, plays a critical role in the regulation of myosin II-dependent in signals that prevent apoptosis. Thus our studies have provided important new information on the role of myosin contractility in adhesion-dependent cell signaling and the potential of inhibiting signal transduction pathways dependent on actomyosin contractility as a therapeutic target and adjuvant for the treatment of breast cancer, as well as other cancers. We are continuing these studies to determine which signaling molecules are downstream of myosin II. These studies will no doubt provide important new insights into the molecular basis of metastatsis.

**REFERENCES:**

**Brown, S.S., and Spudich, J.A.** (1981). Mechanism of action of cytochalasin: evidence that it binds to actin filament ends. J. Cell Biol. 88, 487-491.

**Frisch, S.M., and Francis, H.** (1994). Disruption of epithelial cell-matrix interactions induces apoptosis. J. Cell Biol. 124, 619-626.

**Gimona. M., Kazzaz, J., and Helfman, D.M.** (1996) Forced expression of tropomyosin 2 and 3 in vi-Ki-ras-transformed fibroblasts results in distinct phenotypic effects. Proc. Natl. Acad. Sci. USA 93, 9618-9623.

**Helfman, D.M., Levy, E., Berthier, C., Shtutman, M. Elbaum, and Bershadsky, A.D.** (1997). Block of cell contractility by caldesmon interferes with the rho-dependent formation of focal adhesions. ASCB Meeting, Washington, D.C. Molec. Biol. Cell 8, 415a.

**Helfman, D.M., Levy, E.T., Berthier, C., Shtutman, M. Elbaum, M., and Bershadsky, A.D.** (1999). Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions, Mol. Biol. Cell 10, 3097-3112.

**Qiu, R.-G., Chen, J., McCormick, F., and Symons, M.** (1995). A role for rho in ras transformation. Proc. Natl. Acad. Sci. USA. 92, 11781-11785.

**Thornberry, N.A.** (1998) Caspases: enemies within. Science 281, 1321-1316.

**Yoshioka, K., Matsumura, F., Akedo, H., and Itoh, K.** (1998). Small GTP-binding protein rho stimulates the actomyosin system, leading to invasion of tumor cells. J. Biol. Chem. 273, 5146-5154.

**Zhong, G.C., Kinch, M.S., and Burridge, K.** (1997). Rho-stimulated contractility contributes to the fibroblastic phenotype of Ras-transformed epithelial cells. Mol. Biol. Cell 8, 2329-44.

**APPENDIX:**

**Cytoskeletal changes in cell transformation and tumorigenesis.** Geraldine Pawlak and David M. Helfman. *Current Opinion in Genetics & Development* 2001, 11:41-47.

# Cytoskeletal changes in cell transformation and tumorigenesis

Geraldine Pawlak* and David M Helfman†

Research during the past couple of years has provided important new information as to how the actin cytoskeleton contributes to growth control in both normal and transformed cells. The cytoskeleton can no longer be viewed as simply a structural framework playing a role in cell shape and motile events such as cell movement, intracellular transport, contractile-ring formation and chromosome movement. More recent experiments show that the cytoskeleton plays a critical role in the regulation of various cellular processes linked to transformation including proliferation, contact inhibition, anchorage-independent cell growth, and apoptosis.

**Addresses**
Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724, USA
*e-mail: pawlak@cshl.org
†e-mail: helfman@cshl.org
Correspondence: David M Helfman

**Current Opinion in Genetics & Development** 2001, 11:41–47

0959-437X/01/$ – see front matter


**Abbreviations**

| | |
|---|---|
| **ECM** | extracellular matrix |
| **ERM** | ezrin/radixin/moesin |
| **GEF** | guanine nucleotide exchange factor |
| **MAPK** | mitogen-activated protein kinase |
| **MLC** | myosin light chain |
| **PAK** | p21-activated kinase |
| **ROCK** | Rho kinase |
| **Tiam1** | T-lymphoma invasion and metastasis 1 |
| **TM** | tropomyosin |

## Introduction

Transformation of cells in tissue culture results in a variety of cellular changes including alterations in serum- and adhesion-dependent cell growth, loss of contact inhibition, changes in adhesiveness, motility, morphology, and organization of the cytoskeleton. Disruption of actin filaments and a decrease in focal adhesions are common features following transformation of cells by various oncogenes. These changes in microfilament structure are highly related to both anchorage-independent growth and cellular tumorigenicity, suggesting fundamental roles for actin filaments in oncogenic transformation. The alterations in actin filament structure were found to correlate with decreased expression of various cytoskeleton proteins [1]. Subsequent experiments were carried out in order to determine if the decrease in the expression of specific actin filament-associated proteins contribute to the transformed phenotype. These studies demonstrated that the re-expression of these proteins, via transfection, frequently suppresses many features of transformation including restored formation of microfilament bundles, focal adhesions, contact-inhibited cell growth, inability to grow on soft agar, and suppression of tumorigenicity in nude mice. These studies include the actin-associated proteins α-actinin [2,3], gelsolin [4], profilin [5], vinculin [6] and tropomyosin (TM) [7,8•,9–12]. Although the mechanisms by which these proteins contribute to growth control remain to be fully elucidated, these studies demonstrate that changes in the expression of specific structural components of the actin cytoskeleton can contribute to transformation. In addition, much effort has been directed towards understanding how the Ras/Rho family of GTPases (Rho, rac and cdc42) regulates actin-filament assemblies in both normal and transformed cells [13,14•,15,16,17•]. Here, we review recent studies that shed light on how structural components of the actin cytoskeleton contribute to changes in microfilament assembly and how changes in these proteins might contribute to alterations in growth control and tumorigenesis.

## Actin-filament assemblies, small GTPases and transformation

Oncogenic activity of small GTPases can target actin-containing structures by several mechanisms including alterations in the expression of cytoskeletal proteins (e.g. TM) or interaction with cytoskeletal proteins (e.g. ezrin and ankyrin). Studies of Ras-transformed cells have raised some intriguing issues regarding the actin cytoskeleton and the role of Rho family proteins in transformation. Rho is thought to act downstream of Ras but whereas Rho activation is known to promote the development of stress fibers and focal adhesions, Ras-transformed fibroblasts often exhibit a loss of these structural elements. Interestingly, activated RhoA can restore stress fibers in Ras-transformed cells [18]. Conversely, inhibition of Rho kinase (ROCK) — an effector of RhoA that is involved in stress-fiber formation — can block Ras transformation [19]. One possibility is that the frequently observed downregulation in the expression of various actin filament associated proteins is required for transformation by Ras and perhaps other oncogenes. The TMs are a family of rod-shaped proteins that bind along both grooves of filamentous actin [20]. TMs bound along actin filaments can stabilize filaments by protecting them from the action of severing proteins. Decreased expression of high molecular weight TM isoforms (284 amino acids) is found in fibroblasts transformed by various oncogenes, carcinogens, DNA and RNA tumor viruses [20]. In addition to studies using cells in culture, changes in TM expression have been found in human prostate cancer and breast cancer [21–23]. More recent studies [24•–26•] using microarrays have reported changes in TM expression and other components of the actin cytoskeleton in metastatic melanoma cells and MYC-transformed cells. At present, the mechanism for downregulation of TM expression in *ras*-transformed cells is not known. In one study, the *ras*-induced downregulation of TM was found to be Raf-mediated, but

MEK-independent because treatment with the MEK1 inhibitor PD98059 had little effect on TM levels, suggesting that a novel pathway exists downstream of Raf which may play an important role in the regulation of the cytoskeleton [27]. In a study of c-Jun transformed cells, however, the downregulation of TM was reversed following treatment of cells with the MEK1 inhibitor PD98059 [28]. Clearly more work will be required to understand the pathways leading to the decreased synthesis of TM. Properties of Ras-transformed and *src*-transformed cells were reverted to normal following forced expression of specific high molecular weight TMs, including restored formation of microfilament bundles, contact-inhibited cell growth, inability to grow on soft agar, and suppression of tumorigenicity in nude mice. [7,8•,10,12]. How forced expression of TM can lead to suppression of the transformed phenotype is still not understood. In addition to a role in stabilizing actin filaments, TM might play a role in the regulation of myosin II function and adhesion-dependent signaling (see below).

A role for ezrin/radixin/moesin (ERM) proteins in transformation has been reported [29••,30••]. Early studies implicated ERM proteins in transformation as they were found to be overexpressed upon transformation [31,32] and in metastatic cell lines [33,34]. ERM proteins form crosslinks between cortical actin filaments and the plasma membrane. They play a role in formation of microvilli, cell-adhesion sites, lamellipodia and contractile rings during cytokinesis. ERM proteins are also structurally related to the tumor-suppressor protein merlin/schwannomin, which is involved in neurofibromatosis type II [35•]. Overexpression of merlin/schwannomin in 3T3 cells suppresses the Ras-transformed phenotype (i.e. anchorage-independent growth on soft agar and restores contact inhibition of cell growth) [36,37]. Recently, the TSC1 tumor-suppressor hamartin was found to interact directly with ERM proteins [29••]. The interaction of hamartin with ERM proteins is required for the activation of Rho by serum or lysophosphatidic acid, which normally results in the assembly of actin stress fibers and formation of focal adhesions. The loss of hamartin is thought to result in disruption of cell adhesion to the extracellular matrix (ECM), which may initiate the development of TSC hamartomas. These studies also indicate that a Rho-mediated signaling pathway regulating cell adhesion may constitute a rate-limiting step in tumor formation. Whether hamartin activates Rho through the activation of a guanine nucleotide exchange factor (GEF) or by suppression of the activity of a guanine nucleotide dissociation inhibitor remains to be determined. In a related study ezrin was found to be required for ROCK-mediated transformation by the Net and Dbl oncogenes which are RhoA GEFs [30••]. ROCK was found to phosphorylate ezrin on threonine 567 and mutation of this site to alanine inhibited ROCK-induced relocalization of ezrin to actin-containing structures, and also inhibited RhoA-mediated contractility and focal-adhesion formation. Interestingly, ezrin T567A (but not wild-type ezrin) specifically inhibited transformation by the RhoGEFs Dbl and Net but not by v-*src*, suggesting that ezrin activation is an essential target in transformation induced by these oncogenes. The ezrin T567A also inhibited *ras*-transformation, in agreement with the observation that RhoA-signaling pathways contribute to transformation by Ras [19,38]. This study shows that ezrin-mediated cytoskeletal rearrangements induced by ROCK are required for RhoA- and RhoGEF-induced transformation.

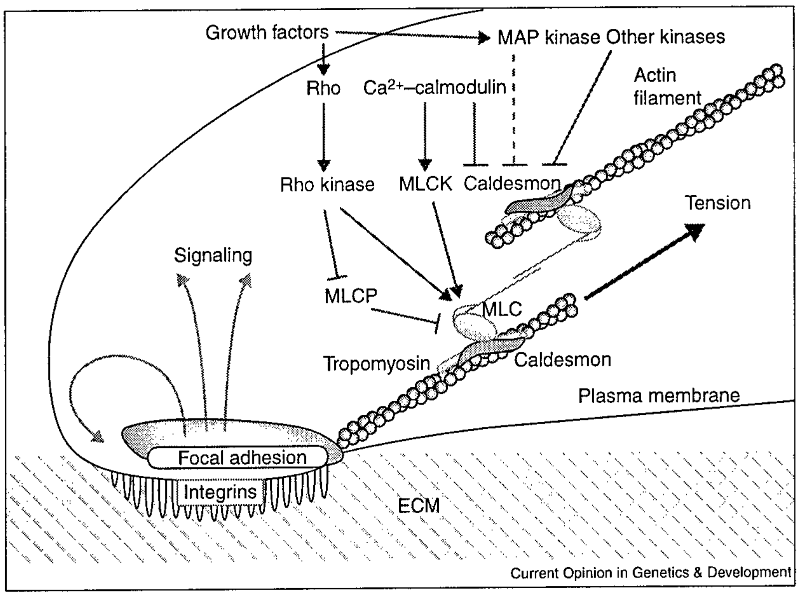
Tiam1 (T-lymphoma invasion and metastasis 1) is one of the GEFs for Rac1, whose expression was implicated in metastasis [39]. Recent studies of the mechanism of Tiam1–Rac1 signaling in metastatic breast tumor cells reveal that Tiam1 interacts with ankyrin [40••]. Ankyrin belongs to a family of cytoskeletal proteins that couple a variety of membrane-spanning cell surface proteins to the spectrin–actin cytoskeleton on the cytoplasmic surface of the plasma membrane [41•]. Importantly, the binding of Tiam1 to ankyrin activates the GDP/GTP exchange on Rac1. This study suggests that the interaction of Tiam1 with ankyrin plays a critical role in regulating Rac1-activated oncogenic signaling and cytoskeleton-mediated metastatic breast tumor cell progression.

## Actomyosin contractility in adhesion-dependent signal transduction

Cell adhesion involves dynamic interactions between the ECM, various transmembrane adhesion receptors, and the actin cytoskeleton. These interactions play a critical role in the regulation of cell growth, differentiation and survival, as well as cell shape and motility. During tumor cell progression a number of cellular features are altered including acquisition of unregulated proliferation and the ability of cells to grow outside their local environment (metastasize)-the latter feature being a major factor in the development of cancer and a problem in its treatment. The loss of adhesion-responsive regulation of cell growth is a characteristic feature that distinguishes transformed cells from normal cells. Cell growth often becomes independent of growth factors and adhesion (anchorage-independent) in transformed cells, as exemplified by their ability to grow on soft agar.

Adhesion of cells to the ECM via transmembrane receptors of the integrin family induces a rapid sequence of events including structural alterations (formation of focal contacts and microfilament bundles), as well as signal transduction (e.g. tyrosine phosphorylation of focal adhesion kinase [FAK], paxillin and p130cas), ultimately resulting in regulation of the cell-cycle machinery [42,43••]. Critical cellular components involved in this adhesion-dependent signaling are specialized structures called focal adhesions. At these sites, clustered integrins span the plasma membrane and interact extracellularly with components of the ECM, and on the cytoplasmic side with cytoskeletal proteins that function in the attachment of bundles of actin filaments (stress fibers) to these regions. Proteins involved in signal transduction are also concentrated at these sites. Although certain steps in the signaling cascade are well characterized,

**Figure 1**




Proposed model for the involvement of actin filaments, myosin II, TM and caldesmon in the regulation of cell contractility and adhesion-dependent signaling. Actomyosin contractility in non-muscle cells involves a number of structural components including actin, myosin II, TM, and caldesmon. Tension on integrins at focal adhesions developed by the actin system affects adhesion-dependent signaling. This signaling, in turn, promotes further assembly of the focal adhesions and also induces downstream events such as cell proliferation and suppression of apoptosis. Tension is positively regulated by MLC kinase (MLCK) and Rho via Rho-kinase and negatively regulated by caldesmon. Caldesmon is potentially regulated by a number of kinases (see main text for discussion). Inhibition of the actin-activated myosin ATPase activity by caldesmon is dependent on TM. For simplicity, isolated copies of TM and caldesmon are shown, though in fact they are localized along the actin filaments. MLCP, MLC phosphatase.

the complete mechanism of adhesion-dependent signaling is not fully understood. In addition to attachment to the appropriate ECM, the activation of adhesion-dependent signaling requires co-stimulation by soluble ligands (growth factors), whose effects are mediated by the small GTP-binding protein Rho [13,44]. One target of Rho is activation of ROCK, which phosphorylates, and thereby inactivates myosin light chain (MLC) phosphatase resulting in activation of myosin contractility [44,45]. Thus, activation of myosin II contractility is a target during adhesion-dependent signaling.

Recent studies have demonstrated that activation of myosin II (i.e. actomyosin contractility), is a critical step in adhesion-dependent signaling via its effects on formation of focal adhesions [46–48,49••]. Pharmacological agents that block actomyosin contractility inhibit Rho-induced formation of stress fibers and focal adhesions, as well as phosphorylation of FAK and paxillin [47]. More recent results [49••] show that overexpression of caldesmon, a protein involved in inhibition of actomyosin contractility, blocks Rho-induced formation of stress fibers and tyrosine phosphorylation of focal adhesions. The mechanism by which increased contractility results in formation of stress fibers, focal adhesions and elevated tyrosine phosphorylation is not fully understood. One hypothesis is that integrins are normally loosely associated within the cell membrane but stimulation of myosin contractility results in bundling of the actin, generating tension that would aggregate the integrins [47,50]. Integrin clustering activates FAK, leading to FAK autophosphorylation and recruitment to the developing focal adhesion of various signaling proteins, which would account for the increase in tyrosine phosphorylation observed in Rho-stimulated cells [51,52]. These studies demonstrate that the actin cytoskeleton plays a key role in cell signaling.

Most studies of the regulation of nonmuscle contractility and its relationship to adhesion-dependent signaling have focused on the role of MLC phosphorylation and its upstream regulators. The observation that activation of actomyosin contractility is an essential step for adhesion-dependent signal transduction suggests a mechanism by which changes in actin-filament-associated proteins could contribute to aberrant growth control (Figure 1). In principle, changes in the levels of cellular proteins involved in the regulation of myosin function observed in transformed cells could lead to activation of actomyosin contractility and contribute to activation of the downstream signaling pathways that are required for cell growth. TM is a natural partner of caldesmon in its regulation of actomyosin-based contractility. *In vitro* studies [53] have demonstrated differences in the abilities of smooth muscle and skeletal muscle TMs to act cooperatively with caldesmon to inhibit myosin II function. On the basis of the results demonstrating an ability of caldesmon to regulate adhesion-dependent signaling [49••], interaction of caldesmon with specific TMs may be important in maintaining the normal signaling functions in the cell. How different nonmuscle TMs will effect the actions of caldesmon on myosin is not known. A loss of specific TMs could contribute to activation of adhesion-dependent signaling pathways as a result of unregulated actomyosin contractility. Caldesmon has been reported to be phosphorylated by various kinases including cdc2, casein kinase II, protein kinase C, cyclic AMP-dependent protein kinase, calcium/calmodulin kinase, mitogen-activated protein kinase (MAPK) and p21-activated kinase (PAK) [54–59].

Phosphorylation of caldesmon may be a target for the regulation of signaling pathways. Studies of caldesmon in smooth muscle have shown it to be phosphorylated by PAK and this phosphorylation was correlated with increased actomyosin contractility [59], although it is as yet unknown if PAK plays a role in the regulation of caldesmon function in nonmuscle cells. In addition, PAK has been implicated in microfilament reorganization and invasiveness of breast-cancer cells [60]. Another line of evidence for caldesmon in signaling and transformation has been suggested in studies showing that caldesmon is tyrosine phosphorylated in a complex with Shc–Grb2–Sos in v-ErbB-transformed cells [61,62,63••]. Clearly, further studies will be required to determine the role played by TM and caldesmon in nonmuscle myosin function and intracellular signaling.

## Apoptosis

The role of the actin cytoskeleton in the execution phase of apoptosis — characterized by morphological changes such as cell rounding, membrane blebbing, and chromatin condensation — is well established [64•]. However, the cytoskeleton can also play a role in the regulation of apoptosis as suggested by the finding that cytoskeletal disruption by cytochalasin D can induce apoptosis [65]. In addition, apoptosis driven by various signals is accompanied by the specific cleavage of actin-associated proteins, including gelsolin [66], fodrin [67], adducin [68] and myosin heavy chain [69]. These studies suggest that disruption of the cytoskeleton is a required step during apoptosis. Modification of the actin cytoskeleton state also impacts upon signals leading to apoptosis: stabilization of actin filaments by jasplakinolide was shown to enhance apoptosis induced by cytokine deprivation [70]. Integrity of the cytoskeleton has been shown to be essential for CD95-induced apoptosis, through an ezrin-mediated association between CD95 and the actin cytoskeleton [71••]. Conversely, cytoskeleton disruption by cytochalasin D accelerates DNA-damage-induced apoptosis [72]. The apparent discrepancies between these studies may reflect cell-type specificity. Much of our understanding of cytoskeleton function is derived from studies of fibroblast cell lines and it is conceivable that the cytoskeleton plays different roles in signal transduction events depending on the cell type. Nonetheless, the actin cytoskeleton appears to play a critical role in the regulation of cell response to apoptotic signals but how it regulates downstream targets remains to be determined.

Disruption of cell–ECM interaction results in a reversible induction of apoptosis — a phenomenon termed 'anoikis' [73–75]. This process is believed to play a critical role in preventing the growth of cells outside their local environment (i.e. metastasis). Conversely, inhibition of anoikis in tumor cells allows them to invade surrounding tissues. Several molecules and signaling pathways induced by interaction of the cell with the ECM have been implicated in the regulation of anoikis [43••], among them FAK, phosphatidylinositol-3-kinase, Akt, nuclear factor (NF)-κB, and MAP kinases. Activation of FAK is a critical component as it prevents anoikis [76]. The downstream effectors remain to be established but recent studies indicate that FAK can suppress p53-mediated apoptosis [77]. Furthermore, p53 serves to monitor survival signals from the ECM/FAK as apoptosis is suppressed by dominant negative p53. In addition to FAK, a second component of focal adhesions, namely CAS, has been implicated in regulation of a poptosis [78,79••]. The CAS proteins — p130Cas, HEF1/Cas-L and Efs/Sin — are a family of docking proteins that contain multiple interaction domains, and are important components of integrin-receptor signaling [80]. Binding of the SH3 domain of p130cas to proline-rich region 1 in FAK was found to be required for prevention of apoptosis on fibronectin following serum withdrawal [78]. The FAK–p130Cas complex was found to activate c-Jun NH2-terminal kinase (JNK) via Ras/Rac1/Pak1/MAPK kinase 4 (MKK4) pathway. In a related study, extracellular-kinase activation (ERK) activation and CAS/Crk coupling are required to prevent apoptosis [79••]. Collectively, these studies demonstrate that cell–matrix interactions play an essential role in preventing apoptosis in normal cells and that this property is abrogated in transformed cells. It still remains to be determined how these pathways interact to regulate cell growth and survival and what role cytoskeletal elements plays in their function. The generation of signals induced by the interaction of cells with the ECM requires the maintenance of an intact cytoskeleton [81]. Furthermore, on the basis of results showing that adhesion-dependent signaling could be modulated by actomyosin contractility, it seems reasonable to predict that further analyses will reveal a pivotal role for the actin cytoskeleton in the regulation of survival signals generated by cell–ECM interactions.

## Conclusions

Studies during the past few years have provided new clues regarding the relationship between the actin cytoskeleton and growth control. The direct interaction with cytoskeletal proteins during the activation of Rho and Rac — as described for hamartin/ezrin and Tiam1/ankyrin — most likely represents a paradigm for the action of other signaling molecules. These studies highlight the importance of the cytoskeleton in the spatial organization of signaling. It will be important to determine if the loss of stress fibers observed in transformed cell acts, in part by affecting the localization of signaling molecules. In addition, the actin cytoskeleton affects the organization and function of adhesive structures such as integrins and cadherins [14•,42], and alterations in the cytoskeleton can impinge on signaling pathways involving these. Cell–cell junctions play an important role in cell proliferation control and studies of the adenomatous polyposis coli highlight the role of junctional proteins involved in these structures [82]. Interestingly, overexpression of β-catenin was found to protect nontransformed epithelial cells from apoptosis, indicating that both integrin- and cadherin-containing structures play a role in the regulation of apoptosis [83].

Elucidating the mechanisms by which the interactions of cells with the ECM both generate and regulate downstream signals that promote cell growth and survival is critical to understanding cancer. Studies of the cytoskeleton will provide information regarding the possibility that these components might be potential targets for rational drug discovery for cancer. The regulation of myosin II and its relationship to adhesion-dependent signaling will require more studies to elucidate what downstream pathways are effected by activation or inhibition of force generation by myosin II motor molecules. In addition to the cytoplasmic events involving activation of myosin II and integrin signaling, it will be of interest to determine what genes are regulated in response to this type of signaling. Such studies will with no doubt provide important new insights into the genes involved in cell proliferation. With the advent of new microarray technologies, analyses of gene expression in various experimental models of cancer, as well as in human cancers, should provide important new insights in identifying specific cytoskeletal targets.

## Acknowledgements

Due to space limitations, the work of many authors who have made valuable contributions to this field was not cited. We wish to thank Laureen Connel, Edward Kim, Alex Rai and Sungwoo Lee for their critical comments and helpful suggestions regarding this manuscript. DM Helfman was supported by grants from the National Cancer Institute (CA83182) and the Army Breast Cancer Program.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Button E, Shapl C, Lawson D: **Actin, its associated proteins and metastasis.** *Cell Motil Cytoskeleton* 1995, **30**:247-251.

2. Gluck U, Kwiatkowski DJ, Ben-Ze'ev A: **Suppression of tumorigenicity in simian virus 40-transformed 3T3 cells transfected with α-actinin.** *Proc Natl Acad Sci USA* 1993, **90**:383-387.

3. Nikolopoulos SN, Spengler BA, Kisselbach K, Evans AE, Biedler JL, Ross RA: **The human non-muscle α-actinin protein encoded by the ACTN4 gene suppresses tumorigenicity of human neuroblastoma cells.** *Oncogene* 2000, **19**:380-386.

4. Tanaka M, Mullaur L, Ogiso Y, Fujita H, Moriya S, Furuuchi K, Harabayashi T, Shinohara N, Koyanagi T, Kuzumaki N: **Gelsolin: a candidate for suppressor of human bladder cancer.** *Cancer Res* 1995, **55**:3228-3232.

5. Janke J, Schluter K, Jandrig B, Theile M, Kolbe K, Arnold W, Grinstein E, Schwartz A, Estevez-Schwarz L, Schlag PM *et al.*: **Suppression of tumorigenicity in breast cancer cells by the microfilament protein profilin 1.** *J Exp Med* 2000, **191**:1675-1685.

6. Rodriguez-Fernandez JL, Geiger B, Salomon D, Sabanay I, Zoller M, Ben-Ze'ev A: **Suppression of tumorigenicity in transformed cells after transfection with vinculin.** *J Cell Biol* 1992, **119**:427-438.

7. Prasad GL, Fuldner RA, Cooper HL: **Expression of transduced tropomyosin 1 cDNA suppresses neoplastic growth of cells transformed by the *ras* oncogene.** *Proc Natl Acad Sci USA* 1993, **90**:7039-7043.

8. • Prasad GL, Masuelli L, Raj MHG, Harindranath N: **Suppression of *src*-induced transformed phenotype by expression of tropomyosin-1.** *Oncogene* 1999, **18**:2027-2031.

This paper shows that re-expression of tropomyosin-1 can suppress the *src*-transformed phenotype, in addition to its previously demonstrated anti-oncogenic effect on Ras-transformed cells. Thus, TM1 is an anti-oncogene of functionally diverse oncogenes and may be considered as a class II tumor suppressor protein.

9. Takenaga K, Masuda A: **Restoration of microfilament bundle organization in v-raf-transformed NRK cells after transduction with tropomyosin 2 cDNA.** *Cancer Lett* 1994, **87**:47-53.

10. Gimona M, Kazzaz J, Helfman DM: **Forced expression of tropomyosin 2 or 3 in v-Ki-ras-transformed fibroblasts results in distinct phenotypic effects.** *Proc Natl Acad Sci USA* 1996, **93**:9618-9623.

11. Braverman RH, Cooper HL, Lee HS, Prasad GL: **Anti-oncogenic effects of tropomyosin: isoform specificity and importance of protein coding sequences.** *Oncogene* 1996, **13**:537-545.

12. Janssen RAJ, Mier JW: **Tropomyosin-2 cDNA lacking the 3′ untranslated region riboregulator induces growth inhibition of v-Ki-ras-transformed fibroblasts.** *Mol Biol Cell* 1997, **8**:897-908.

13. Hall A: **Rho GTPases and the actin cytoskeleton.** *Science* 1998, **279**:509-514.

14. • Schoenwaelder SM, Burridge K: **Bidirectional signaling between the cytoskeleton and integrins.** *Curr Opin Cell Biol* 1999, **11**:274-286.

Signaling pathways that feedback from integrins to the cytoskeleton are described extensively in this review. The role played by myosin-mediated cell contractility is discussed.

15. Hernandez-Alcoceba R, del Peso L, Lacal JC: **The ras family of GTPases in cancer cell invasion.** *Cell Mol Life Sci* 2000, **57**:65-76.

16. Evers EE, Zondag GCM, Malliri A, Price LS, ten Klooster J-P, van der Kammen RA, Collard JG: **Rho family proteins in cell adhesion and cell migration.** *Eur J Cancer* 2000, **36**:1269-1274.

17. • Shields JM, Pruit K, McFall A, Shaub A, Der CJ: **Understanding Ras: 'it ain't over 'til it's over'.** *Trends Cell Biol* 2000, **10**:147-154.

This review highlights unresolved, challenging issues regarding Ras function that have arisen from recent studies originally designed to understand Ras biology, biochemistry, genetics and structure.

18. Izawa I, Amano M, Chihara K, Yamamoto T, Kaibuchi K: **Possible involvement of the inactivation of the Rho-Rho-kinase pathway in oncogenic Ras-induced transformation.** *Oncogene* 1998, **17**:2863-2871.

19. Sahai E, Ishizaki T, Narumiya S, Treisman R: **Transformation mediated by RhoA requires activity of ROCK kinases.** *Curr Biol* 1999, **9**:136-145.

20. Pittenger MF, Kazzaz JA, Helfman DM: **Functional properties of non-muscle tropomyosin isoforms.** *Curr Opin Cell Biol* 1994, **6**:96-104.

21. Bhattacharya B, Prasad GL, Valverius EM, Salomon DS, Cooper HL: **Tropomyosins of human mammary epithelial cells: consistent defects of expression in mammary carcinoma cell lines.** *Cancer Res* 1990, **50**:2105-2112.

22. Franzen B, Linder S, Uryu K, Alaiya AA, Hirano T, Kato H, Auer G: **Expression of tropomyosin isoforms in benign and malignant human breast lesions.** *Brit J Cancer* 1996, **73**:909-913.

23. Wang F-L, Wang Y, Wong W-K, Liu Y, Addivinola J, Liang P, Chen LB, Kantoff PW, Pardee AB: **Two differentially expressed genes in normal human prostate tissue and in carcinoma.** *Cancer Res* 1996, **56**:3634-3637.

24. • Clark EA, Golub TR, Lander ES, Hynes RO: **Genomic analysis of metastasis reveals an essential role for RhoC.** *Nature* 2000, **406**:532-535.

This paper demonstrates how gene-expression profiling, using high-density DNA microarrays, is revolutionizing our approach to studying cancer. Importantly, the three genes identified as consistently upregulated in melanoma-derived metastases – *RhoC, fibronectin* and *thymosin β4* – are all involved in regulation of actomyosin contractility and migration. See also annotations [25•,26•].

25. • Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, Radmacher M, Simon R, Yakhini Z, Ben-Dor A *et al.*: **Molecular classification of cutaneous malignant melanoma by gene expression profiling.** *Nature* 2000, **406**:536-540.

See annotation [24•]. In this study, increased fibronectin expression is correlated with higher invasive capacity (but not RhoC or thymosin β4).

26. • Coller HA, Grandori C, Tamayo P, Colbert T, Lander E, Eisenman RN, Golub TR: **Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in cell growth, cell**

**cycle, signaling, and adhesion.** *Proc Natl Acad Sci USA* 2000, **97**:3260-3265.
See annotation [24•]. The specific changes in gene expression observed in this study suggest new mechanisms for the biological functions of MYC.

27. Janssen RAJ, Veenstra KG, Jonasch P, Jonasch E, Mier JW: **Ras- and Raf-induced down-modulation of non-muscle tropomyosin are MEK-independent.** *J Biol Chem* 1998, **273**:32182-32186.

28. Ljungdahl S, Linder S, Franzen B, Binetruy B, Auer G, Shoshan MC: **Down-regulation of tropomyosin-2 expression in c-Jun-transformed rat fibroblasts involves induction of a MEK1-dependent autocrine loop.** *Cell Growth Diff* 1998, **9**:565-573.

29. •• Lamb RF, Roy C, Diefenbach TJ, Vinters HV, Johnson MW, Jay DG, Hall A: **The TSC1 tumor suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho.** *Nat Cell Biol* 2000, **2**:281-287.
First evidence that misregulation of cell–cell and cell–matrix interactions, through the loss of interaction between the tumor suppressor hamartin and the cytoskeleton-associated protein ezrin, may contribute to the development of hamartomas in individuals carrying *TSC1* mutations.

30. •• Quang CT, Gautreau A, Arpin M, Treisman R: **Ezrin function is required for ROCK-mediated fibroblast transformation by the Net and Dbl oncogenes.** *EMBO J* 2000, **19**:4565-4576.
ERM-mediated cytoskeletal rearrangements induced by ROCK are shown to be required for RhoA- and RhoGEF-induced transformation. This paper shows that phosphorylation of ezrin plays a critical role in transformation by the Net and Dbl oncogenes.

31. Joos KU, Muller R: **Deregulation of genes encoding microfilament-associated proteins during Fos-induced morphological transformation.** *Oncogene* 1995, **10**:603-608.

32. Lamb RF, Ozanne BW, Roy C, McGarry L, Stipp C, Mangeat P, Jay DJ: **Essential function of ezrin in maintenance of cell shape and lamellipodial extension in normal and transformed fibroblasts.** *Curr Biol* 1997, **7**:682-688.

33. Akisawa N, Nishimori I, Iwamura T, Onishi S, Hollingsworth MA: **High levels of ezrin expressed by human pancreatic adenocarcinoma cell lines with high metastatic potential.** *Biochem Biophys Res Commun* 1999, **258**:398-400.

34. Ohtani K, Sakamoto H, Rutherford T, Chen Z, Satoh K, Naftolin F: **Ezrin, a membrane-cytoskeletal linking protein, is involved in the process of invasion in endometrial cancer cells.** *Cancer Lett* 1999, **147**:31-38.

35. • Mangeat P, Roy C, Martin M: **ERM proteins in cell adhesion and membrane dynamics.** *Trends Cell Biol* 1999, **9**:187-192.
An excellent overview of ERM proteins.

36. Tikoo A, Varga M, Ramesh V, Guesella J, Maruta H: **An anti-ras function of neurofibromatosis type 2 gene product (NF2/Merlin).** *J Biol Chem* 1994, **269**:23387-23390.

37. Lutchman M, Rouleau GA: **The neurofibromatosis type 2 gene product, schwannomin, suppresses growth in NIH 3T3 cell.** *Cancer Res* 1995, **55**:2270-2274.

38. Qiu R-G, Chen J, McCormick F, Symons M: **A role for rho in ras transformation.** *Proc Natl Acad Sci USA* 1995, **92**:11781-11785.

39. Habets GG, Scholtes EH, Zuydgeest D, van der Kammen RA, Stam JC, Berns A, Collard JG: **Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins.** *Cell* 1994, **77**:537-549.

40. •• Bourguignon LYW, Zhu H, Shao L, Chen YW: **Ankyrin–Tiam1 interaction promotes Rac1 signaling and metastatic breast tumor cell invasion and migration.** *J Cell Biol* 2000, **150**:177-191.
Results described in this paper suggest that Tiam1 interaction with the cytoskeletal protein ankyrin promotes Rac GTPase activation and cytoskeletal changes required for metastatic breast tumor cell invasion and migration.

41. • De Matteis MA, Morrow JS: **The role of ankyrin and spectrin in membrane transport and domain formation.** *Curr Opin Cell Biol* 1998, **10**:542-549.
An excellent overview of ankyrin and spectrin.

42. Ben-Ze'ev A, Bershadsky AD: **The role of the cytoskeleton in adhesion-mediated signaling and gene expression.** *Adv Mol Cell Biol* 1997, **24**:125-163.

43. •• Aplin AE, Howe A, Alahari SK, Juliano RL: **Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins.** *Pharmacol Rev* 1998, **50**:197-264.
An extensive review of signal transduction and signal modulation by cell-adhesion receptors. The crucial role played by integrin-mediated interactions with ECM components in growth, differentiation and survival are emphasized.

44. Van Aelst L, D'Souza-Schorey C: **Rho GTPases and signaling networks.** *Genes Dev* 1997, **11**:2295-2322.

45. Kimura H, Ito M, Amano K, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K *et al.*: **Regulation of myosin phosphatase by rho and rho-associated kinase (rho-kinase).** *Science* 1996, **273**:245-248.

46. Bershadsky A, Chausovsky A, Becker E, Lyubimova A, Geiger B: **Involvement of microtubules in the control of adhesion-dependent signal transduction.** *Curr Biol* 1996, **6**:1279-1289.

47. Chrzanowska-Wodnicka M, Burridge K: **Rho-stimulated contractility drives the formation of stress fibers and focal adhesions.** *J Cell Biol* 1996, **133**:1403-1415.

48. Zhang Q, Magnusson MK, Mosher DF: **Lysophosphatidic acid and microtubule-destabilizing agents stimulate fibronectin matrix assembly through rho-dependent actin stress fiber formation and cell contraction.** *Mol Biol Cell* 1997, **8**:1415-1425.

49. •• Helfman DM, Levy ET, Berthier C, Shtutman M, Riveline D, Grosheva I, Lachish-Zalait A, Elbaum M, Bershadsky AD: **Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions.** *Mol Biol Cell* 1999, **10**:3097-3112.
In this paper, it is shown that overexpression of caldesmon inhibits cell contractility and blocks Rho-induced formation of focal adhesions. It further implicates a role for myosin in adhesion-dependent signaling and suggests a role for caldesmon in signal transduction in non-muscle cells.

50. Burridge K, Chrzanowska-Wodnicka M, Zhong C: **Focal adhesion assembly.** *Trends Cell Biol* 1997, **7**:342-347.

51. Kornberg L, Earp HS, Parsons JT, Schaller M, Juliano RL: **Cell adhesions or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase.** *J Biol Chem* 1992, **267**:23439-23442.

52. Miyamoto S, Akiyama SK, Yamada KM: **Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function.** *Science* 1995, **267**:883-885.

53. Marston SB, Redwood CS: **The essential role of tropomyosin in cooperative regulation of smooth muscle thin filament activity by caldesmon.** *J Biol Chem* 1993, **268**:12317-12320.

54. Matsumura F, Yamashiro S: **Caldesmon.** *Curr Opin Cell Biol* 1993, **5**:70-76.

55. Somlyo AP, Somlyo AV: **Signal transduction and regulation in smooth muscle.** *Nature* 1994, **372**:231-236.

56. Franklin MT, Wang CL, Adam LP: **Stretch-dependent activation and desensitization of mitogen-activated protein kinase in carotid arteries.** *Am J Physiol* 1997, **273**:1819-1827.

57. Van Eyk JE, Arrell DK, Foster DB, Strauss JD, Heinonen TYK, Furmaniak-Kazmierczak E, Cote GP, Mak AS: **Different molecular mechanisms for Rho family GTPase-dependent, $Ca^{2+}$-independent contraction of smooth muscle.** *J Biol Chem* 1998, **273**:23433-23499.

58. D'Angelo G, Graceffa P, Wang CLA, Wrangle J, Adam LP: **Mammal-specific, ERK-dependent, caldesmon phosphorylation in smooth muscle.** *J Biol Chem* 1999, **274**:30115-30121.

59. Foster DB, Shen L-H, Kelly J, Thibault P, Van Eyk JE, Mak AS: **Phosphorylation of caldesmon by p21-activated kinase: implications for the $Ca^{2+}$ sensitivity of smooth muscle contraction.** *J Biol Chem* 2000, **275**:1959-1965.

60. Adam L, Vadlamudi R, Mandal M, Chernoff J, Kumar R: **Regulation of microfilament reorganization and invasiveness of breast cancer cells by kinase dead p21-activated kinase-1.** *J Biol Chem* 2000, **275**:12041-12050.

61. McManus MJ, Lingle WL, Salisbury JL, Maihle NJ: **A transformation-associated complex involving tyrosine kinase signal adaptor proteins and caldesmon links v-ErbB signaling to actin stress fiber disassembly.** *Proc Natl Acad Sci USA* 1997, **94**:11351-11356.

62. Wang Z, Danielsen AJ, Maihle NJ, McManus MJ: **Tyrosine phosphorylation of caldesmon is required for the binding to the Shc–Grb2 complex.** *J Biol Chem* 1999, **247**:33807-33813.

63. McManus MJ, Boerner JL, Danielsen AJ, Wang Z, Matsumura F,
•• Maihle NJ: **An oncogenic epidermal growth factor receptor signals via a p21-activated kinase–caldesmon–myosin phosphotyrosine complex.** *J Biol Chem* 2000, **275**:35328-35334.

This study suggests the stimulation of novel, transformation-specific signaling networks arising from altered substrate specificity of the p21 kinase as a mechanism of oncogenic signaling. This finding implicates phosphorylation of caldesmon in the altered regulation of myosin function in transformation.

64. Mills JC, Stone NL, Pittman RM: **Extranuclear apoptosis: the role of**
• **the cytoplasm in the execution phase.** *J Cell Biol* 1999, **146**:703-707.

Reviews involvement of the cytoskeleton in apoptosis.

65. Rosen K, Rak J, Leung T, Dean NM, Kerbel RS, Filmus J: **Activation of ras prevents downregulation of Bcl-X(L) triggered by detachment from the extracellular matrix: a mechanism of ras-induced resistance to anoikis in intestinal epithelial cells.** *J Cell Biol* 2000, **149**:447-456.

66. Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Koths K, Kwiatkowski DJ, Williams LT: **Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis.** *Science* 1997, **278**:294-298.

67. Janicke RU, Ng P, Sprengart ML, Porter AG: **Caspase-3 is required for alpha-fodrin cleavage but dispensable for cleavage of other death substrates in apoptosis.** *J Biol Chem* 1998, **273**:15540-15545.

68. Van de Water B, Tijdens IB, Verbrugge A, Huigsloot M, Dihal AA, Stevens JL, Jaken S, Mulder GJ: **Cleavage of the actin-capping protein alpha-adducin at asp-ser-asp633-ala by caspase-3 is preceded by its phosphorylation on serine 726 in cisplatin-induced apoptosis of renal epithelial cells.** *J Biol Chem* 2000, **275**:25805-25813.

69. Suarez-Huerta N, Lecocq R, Mosselmans R, Galand P, Dumont JE, Robaye B: **Myosin heavy chain degradation during apoptosis in endothelial cells.** *Cell Prolif* 2000, **33**:101-114.

70. Posey SC, Bierer BE: **Actin stabilization by jasplakinolide enhances apoptosis induced by cytokine deprivation.** *J Biol Chem* 1999, **274**:4259-4265.

71. Parlato S, Giammarioli AM, Logozzi M, Lozupone F, Matarrese P,
•• Luciani F, Falchi M, Malorni W, Fais S: **CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway.** *EMBO J* 2000, **19**:5123-5134.

This paper shows that CD95 co-localizes and co-immunoprecipitates with ezrin exclusively in cells prone to CD95-mediated apoptosis. The CD95 cell membrane polarization, induced by an ezrin-mediated association with the actin cytoskeleton, is a key intracellular mechanism in rendering human T lymphocytes susceptible to the CD95-mediated apoptosis.

72. Yamazaki Y, Tsuruga M, Zhou D, Fujita Y, Shang X, Dang Y, Kawasaki K, Oka S: **Cytoskeletal disruption accelerates caspase-3 activation and alters the intracellular membrane reorganization in DNA damage-induced apoptosis.** *Exp Cell Res* 2000, **259**:64-78.

73. Meredith JE Jr, Fazeli B, Schwartz MA: **The extracellular matrix as a survival factor.** *Mol Biol Cell* 1993, **4**:953-961.

74. Re F, Zanetti A, Sironi M, Polentarutti N, Lanfrancone L, Dejana E, Colotta F: **Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells.** *J Cell Biol* 1994, **127**:537-546.

75. Frisch SM, Ruoslahti E: **Integrins and anoikis.** *Curr Opin Cell Biol* 1997, **9**:701-706.

76. Frisch SM, Vuori K, Ruoslahti E, Chan-Hui P-Y: **Control of adhesion-dependent cell survival by focal adhesion kinase.** *J Cell Biol* 1996, **134**:793-799.

77. Ilic D, Almeida EA, Schlaepfer DD, Dazin P, Aizawa S, Damsky CH: **Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis.** *J Cell Biol* 1998, **143**:547-560.

78. Almeida EAC, Ilic D, Han Q, Hauck CR, Jin F, Kawakatsu H, Schlaepfer DD, Damsky CH: **Matrix survival signaling: from fibronectin via focal adhesion kinase to c-Jun $NH_2$-terminal kinase.** *J Cell Biol* 2000, **149**:741-754.

79. Cho SY, Klemke RL: **Extracellular-regulated kinase activation and**
•• **CAS/Crk coupling regulate cell migration and suppress apoptosis during invasion of the extracellular matrix.** *J Cell Biol* 2000, **149**:223-236.

Molecular evidence is provided that, during invasion of the ECM, cells coordinately regulate migration and survival mechanisms through ERK activation and CAS/Crk coupling.

80. O'Neill GM, Fashena SJ, Golemis EA: **Integrin signalling: a new Cas(t) of characters enters the stage.** *Trends Cell Biol* 2000, **10**:111-119.

81. Clark EA, Brugge JS: **Integrins and signal transduction pathways: the road taken.** *Science* 1995, **268**:233-239.

82. Morin P: **β-catenin signaling and cancer.** *Bioessays* 1999, **21**:1021-1030.

83. Orford K, Orford CC, Byers SW: **Exogenous expression of beta-catenin regulates contact inhibition, anchorage-independent growth, anoikis, and radiation-induced cell cycle arrest.** *J Cell Biol* 1999, **146**:855-868.